

# Attraction of Douglas-fir beetle, spruce beetle and a bark beetle predator (Coleoptera: Scolytidae and Cleridae) to enantiomers of frontalin

B. STAFFAN LINDGREN

PHERO TECH INC., 7572 PROGRESS WAY, RR #5, DELTA, B.C. V4G 1E9, CANADA

## ABSTRACT

In three separate experiments, Douglas-fir beetles, *Dendroctonus pseudotsugae* Hopkins, preferred traps baited with either (*S*)-(-)- or racemic (*R,S*)-(±)-frontalin over those baited with the (*R*)-(+) -enantiomer. Spruce beetles, *D. rufipennis* (Kirby), appeared to be attracted equally to both the (*S*)-(-)- and (*R*)-(+) -enantiomers, but low catches and high variance made interpretation of the data tenuous. For both species racemic frontalin was as attractive as the preferred enantiomer alone. The bark beetle predator, *Thanasimus undatulus* (Say), was attracted preferentially to (*S*)-(-)-frontalin over (*R*)-(+) - or (*R,S*)-(±)-frontalin in a Douglas-fir stand, while both enantiomers were equally attractive in a spruce stand.

Additional keywords: *Dendroctonus pseudotsugae*, *Dendroctonus rufipennis*, semiochemical, kairomones, *Thanasimus undatulus*, predator, trapping

## INTRODUCTION

Biological activity of semiochemicals on insects may be maximal to particular enantiomeric blends or restricted to single enantiomers. For example, the ambrosia beetle, *Gnathotrichus retusus* (LeConte), responds to (*S*)-(+) -sulcatol, whereas the presence of (*R*)-(-)-sulcatol is inhibitory (Borden et al. 1980a). Similarly the pine engraver, *Ips pini* (Say), is attracted by (*R*)-(-)-ipsdienol, but inhibited by (*S*)-(+) - ipsdienol in California (Birch et al. 1980). The striped ambrosia beetle, *Trypodendron lineatum* (Olivier), produces and responds to 1(*R*),4(*S*),5(*R*),7(*R*)-(+) -lineatin, whereas the 1(*S*),4(*R*),5(*S*),7(*S*)-(-)-enantiomer is inert (Borden et al. 1980b).

Numerous studies on the chemical ecology of the Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins, and the spruce beetle, *D. rufipennis* (Kirby), have been conducted over the last two decades. Both species produce 1-methylcyclohex-2-en-1-ol (MCOL) and 3-methylcyclohex-2-en-1-one (MCH), and 1,5-dimethyl-6,8- dioxabicyclo[3.2.1]octane (frontalin) (Kinzer et al. 1971; Libbey et al. 1983; Gries et al. 1988; G. Gries<sup>1</sup>, pers. comm.). Both species also exhibit enantiospecific response to MCOL (Lindgren et al. 1992; J.H. Borden<sup>1</sup>, pers. comm.) However, there is no published information on the response by these beetles to enantiomers of frontalin. Thus, the objective of this study was to determine the response of Douglas-fir beetles and spruce beetles to the two frontalin enantiomers alone and in combination.

## MATERIALS AND METHODS

Enantiomers of frontalin (chemical purity >97 %; optical purity 97 % for both enantiomers) were purchased from Simon Fraser University. Lures consisted of frontalin-filled capillaries (45 × 1 mm i.d.) placed in 400 μl polyethylene Eppendorf centrifuge tubes. A 4 mm-diameter hole was cut in the side of each Eppendorf, and the devices suspended in Lindgren funnel traps so that the hole faced downward. In this manner entry of rainwater into the Eppendorf tubes was minimized. The release rate of each enantiomer was estimated at 0.5 mg/24 h @ 24 °C by measuring the drop of the meniscus.

Treatments in all experiments consisted of: (1) (*R*)-(-)-frontalin, (2) (*S*)-(+) -frontalin, and (3) (*R*)-(-)- and (*S*)-(+) -frontalin (one capillary each). In this manner the release rate of each enantiomer was held constant among treatments. In the first experiment, an unbaited control was also included as a fourth treatment. All experiments utilized 8-unit multiple-

funnel traps (Lindgren 1983) (Phero Tech Inc. Delta, B.C.). A 2 × 2 cm piece of dichlorvos-impregnated wax bar was placed in the collection jar of each trap to prevent predatory beetles and ants from destroying the captured bark beetles.

#### Douglas-fir beetle.

The first of three experiments was conducted at the University of British Columbia Research Forest, Maple Ridge, B.C., as a three-block, randomized complete block design experiment, with two time replicates, May 4-11, and May 11-18, 1984. Treatment positions were rerandomized for the second time replicate. The second experiment was conducted at the Manning Creek Forest Road, about 30 km NW of Merritt, B.C., as a seven-block, randomized complete block design experiment May 7-20, 1985. The third experiment was conducted at the Manning Creek Forest Road as a four-block, randomized complete block design experiment, with two time replicates, May 24-27 and May 27-June 4, 1985. Treatment positions were rerandomized for the second time replicate. Captured insects were collected and stored in a freezer until counted and their sex determined (Jantz and Johnsey 1964; Lyon 1958).

#### Spruce beetle.

Two seven-block randomized complete block design experiments were conducted along the Miner Creek Forest Road, about 30 km SW of Merritt, B.C., June 7-18, and June 18-21, 1985. Captured insects were collected, stored and their sex determined as described above.

#### Statistical Analyses.

The data were subjected to analysis of variance ( $\alpha = 0.05$ ), and the means separated by Tukey's Test ( $\alpha = 0.05$ ). All data were transformed as  $x' = \log_{10}(x + 1)$  to remove heterogeneity of variances before analysis. Proportion data in the third Douglas-fir beetle experiment were transformed as  $x' = \arcsin \sqrt{p}$ , where  $p$  is a proportion, and 0 was replaced by  $1/4n$  and 1 by  $1 - (1/4n)$  (Zar 1984). The first and third Douglas-fir beetle experiments, and the spruce beetle experiments, were analyzed as replicated randomized complete blocks.

Table 1

Response by Douglas-fir beetles to Lindgren funnel traps baited with enantiomers of frontalin. Malcolm Knapp Research Forest, Maple Ridge, B.C., 1984 ( $n = 6$ ).

TREATMENT	MEAN NUMBER ( $\pm$ SD) DOUGLAS-FIR BEETLES CAPTURED <sup>a</sup>
UNBAITED CONTROL	0.0 ( $\pm$ 0.0)a
(R)-(+)-FRONTALIN	0.2 ( $\pm$ 0.4)ab
(S)-(-)-FRONTALIN	2.0 ( $\pm$ 2.1)b
(R,S)-( $\pm$ )-FRONTALIN	1.8 ( $\pm$ 1.8)b

<sup>a</sup>Means followed by the same letter not significantly different, analysis of variance and Tukey's test ( $\alpha = 0.05$ )

## RESULTS AND DISCUSSION

**Douglas-fir beetle.** The data analyses from the first experiment, which was conducted in the coastal Douglas-fir zone, indicated that (R)-(-)-frontalin is the attractive enantiomer (Table 1). Although the (S)-(+)-enantiomer was not significantly different at the stated probability level from any other treatment in this experiment, the Tukey HSD probability was  $p = 0.051$  and  $p = 0.055$  when comparing (S)-(+)-frontalin to (R)-(-)- and (R,S)-( $\pm$ )-frontalin, respectively. The two experiments conducted in the interior Douglas-fir zone showed clearly that (R)-(-)-frontalin is the attractive enantiomer for male Douglas-fir beetles, while the (S)-(+)-enantiomer appears to be relatively inactive (Tables 2-3). Thus, male Douglas-fir beetles in both the coastal and interior Douglas-fir zones responded similarly to frontalin enantiomers. Female Douglas-fir beetles were attracted mainly to the (R)-(-)-enantiomer in both the second and third experiment (Tables 2-3). The treatment effect for female catch in the second experiment approached significance ( $p = 0.079$ ), and was highly significant in the

third experiment. There was no treatment effect on sex ratio, expressed as proportion of females, in the third experiment (Table 3). Only two females were captured in the first experiment, both of which responded to traps baited with both enantiomers. Traps baited with both enantiomers tended to capture the highest numbers of beetles of both sexes in all three experiments, indicating that (*S*)-(+)-frontalin may have some activity.

**Table 2**

Response by Douglas-fir beetles and the clerid predator *Thanasimus undatulus* to Lindgren funnel traps baited with enantiomers of frontalin. Manning Creek Road, Merritt Forest District, B.C., May 7-20, 1985 (n = 7).

TREATMENT	MEAN NUMBER ( $\pm$ SD) DOUGLAS-FIR BEETLES AND CLERIDS CAPTURED <sup>a</sup>			
	MALES	FEMALES	TOTAL	CLERIDS
( <i>R</i> )-(+)-FRONTALIN	1.0a ( $\pm$ 1.2)	0.1a ( $\pm$ 0.4)	1.1a ( $\pm$ 1.2)	0.1a ( $\pm$ 0.4)
( <i>S</i> )-(-)-FRONTALIN	16.7b ( $\pm$ 21.2)	3.3a ( $\pm$ 5.7)	20.0b ( $\pm$ 26.7)	2.9b ( $\pm$ 2.6)
( <i>R,S</i> )-( $\pm$ )-FRONTALIN	12.0b ( $\pm$ 11.5)	4.1a ( $\pm$ 4.2)	16.1b ( $\pm$ 15.6)	0.6a ( $\pm$ 0.8)

<sup>a</sup>Means followed by the same letter not significantly different, analysis of variance and Tukey's test ( $\alpha = 0.05$ ).

**Table 3**

Response by Douglas-fir beetles to Lindgren funnel traps baited with enantiomers of frontalin. Manning Creek Road, Merritt Forest District, B.C., May 20-27, 1985 (n = 8).

TREATMENT	MEAN NUMBER ( $\pm$ SD) DOUGLAS- FIR BEETLES CAPTURED <sup>a</sup>			PERCENT FEMALES
	MALES	FEMALES	TOTAL	
( <i>R</i> )-(+)-FRONTALIN	7.5a ( $\pm$ 6.0)	3.8a ( $\pm$ 3.2)	11.3a ( $\pm$ 8.1)	37.4a ( $\pm$ 31.8)
( <i>S</i> )-(-)-FRONTALIN	32.6b ( $\pm$ 39.8)	15.9b ( $\pm$ 19.3)	48.5b ( $\pm$ 59.0)	32.6a ( $\pm$ 8.1)
( <i>R,S</i> )-( $\pm$ )-FRONTALIN	43.6b ( $\pm$ 30.3)	21.1b ( $\pm$ 20.7)	64.8b ( $\pm$ 50.3)	28.9a ( $\pm$ 6.7)

<sup>a</sup>Means followed by the same letter not significantly different, analysis of variance and Tukey's test ( $\alpha = 0.05$ ).

**Spruce beetle.** Catches of spruce beetles were extremely low and variable (Table 4). Very few insects were captured by any treatment, although one trap baited with (*S*)-(+)-frontalin captured 54 beetles in the first experiment. There were no significant treatment effects, and no interactions, in these experiments. Based on these limited data, it appears that spruce beetles respond to both enantiomers. Further experiments are needed to confirm this, as well as to determine geographic variation in the response.

**Clerid beetles,** *Thanasimus undatulus* (Say), were captured in sufficient numbers for statistical analysis in the second Douglas-fir beetle experiment and in the spruce beetle experiment. Significantly more clerids were captured in the traps baited with (*R*)-(-)-frontalin than to either of the treatments containing (*S*)-(+)-frontalin in the Douglas-fir beetle experiment (Table 2), whereas there were no significant differences among the treatments in the spruce beetle experiments. This may indicate some level of behavioral or physiological adaptation, or possibly genetic selection, in clerids predominantly responding to kairomones from a single prey species. Herms et al. (1991) suggested that the related *Thanasimus dubius* (F.) may select for changes in the pheromone system of its prey, *Ips pini*

(Say), which both exhibited considerable inter- and intrapopulational variation in their response to enantiomers of ipsdienol. However, in the experiments reported here the numbers of clerids captured were low, so that additional experiments would be needed to be certain that clerid response to their prey kairomones are enantiospecific, and if such specificity is tied to the pheromone production of its prey.

**Table 4**

Response by spruce beetles and the clerid predator *Thanasimus undatulus* to Lindgren funnel traps baited with enantiomers of frontalin. Miner Creek Road, Merritt Forest District, B.C., June 7-21, 1985 (n = 14).

TREATMENT	MEAN NUMBER ( $\pm$ SD) SPRUCE BEETLES AND CLERIDS CAPTURED <sup>a</sup>			
	MALES	FEMALES	TOTAL	CLERIDS
(R)-(+)-FRONTALIN	2.9 ( $\pm$ 7.6)	2.7 ( $\pm$ 6.6)	5.6 ( $\pm$ 14.1)	2.0 ( $\pm$ 3.0)
(S)-(-)-FRONTALIN	0.8 ( $\pm$ 1.0)	1.1 ( $\pm$ 1.7)	1.9 ( $\pm$ 2.3)	1.6 ( $\pm$ 1.8)
(R,S)-( $\pm$ )-FRONTALIN	1.5 ( $\pm$ 2.1)	1.5 ( $\pm$ 2.7)	3.0 ( $\pm$ 4.6)	4.3 ( $\pm$ 6.8)

<sup>a</sup>There were no significant differences among treatments, analysis of variance ( $\alpha = 0.05$ )

If the lack of treatment effects in the spruce beetle experiments is real, enantiospecific responses to frontalin may be one of the mechanisms whereby these bark beetles maintain species segregation. However, interspecific cross attraction to semiochemicals produced from infested logs has been demonstrated for these species (Chapman and Dyer 1969). For both species, synthetic racemic frontalin can be used in management applications, since it is equally attractive as either enantiomer.

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### NOTE

<sup>1</sup> Department of Biological Sciences, Simon Fraser University, Burnaby, B.C.

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